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Comparative Study for the Effect of Biofertilizers and Chemical Fertilizers on Soybean Oil Content and its Potential for Biodiesel Production

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Abstract. The present study makes comparative evaluation of biofertilizers (brands Biopower and Biozote) and chemical fertilizers (urea and diamonium phosphate (DAP)) on yield and the quality of soybean cv.NARC-1. Significant increase in number of pods per plant, seed oil content and specific gravity of oil was observed in case of chemical fertilizer treatment. All the treatments decreased the acid value and free fatty acid (oleic acid) content of oil, maximum reduction being in the case of Biopower treatment. Biopower treated plant seed oil exhibited higher refractive index and maximum conversion to methyl esters/biodiesel.

Keywords: biodiesel, biofertilizers, soybean oil, chemical fertilizers

Introduction

Alternative fuels produced from renewable feedstock resources are gaining popularity these days. Biodiesel is one of such alternative fuels produced from vegetable oils. The commercial scale production of biodiesel has many socio-economic benefits. The main advantages of biodiesel is its biodegradability, and emission of better quality of exhaust gases, given that all the organic carbon present is photosynthetic in origin (Barnwal and Sharma, 2005).

Chemical fertilizers are unavoidable in increasing crop yield but they have been found to adversely affect ecosystem and are rather expensive with limited resources. On the other hand, biofertlizers are sustainable and environment friendly. Biofertilizers have emerged as a promising component of integrating nutrient supply system in agriculture (Bloemberg *et al.*, 2000). They mainly include nitrogen fixing, phosphate solubilizing and plant growth promoting microorganisms (Goel *et al.*, 1999). Chauhan *et al.* (1995) found that application of biofertilizers markedly increased the pod number and seed yield of *Brassica juncea* L. plants over the non-inoculated ones. Goel *et al.* (1999) reported that inoculation of plants with growth promoting rhizobacteria (PGPR) enhances crop productivity either by making the other nutrients available or protecting plants from pathogenic microorganisms. Zodape (2001) reported that increase in productivity with biofertilizer application is due to microelements and plant growth regulators contained in the fertilizer. Shehata and El-Khawas (2003) reported that oil contents and seed yield in sunflower significantly increased in response to biofertilizer application as compared to the control.

Soybean (*Glycine max* L.) Merrill is among the most important oilseed crops containing 18 to 22 percent oil with 85% unsaturated fatty acids and is widely used for biodiesel production. In view of the limited availability of biodiesel resources, the present investigation was carried out to compare the effects of chemical fertilizers and biofertilizers on the yield and quality of soybean oil pertaining to biodiesel production.

Materials and Methods

The experiment was carried out in complete randomized design in green house at the Department of Plant Sciences, Quaid-e-Azam University, Islamabad. Seeds of soybean cv. NARC-1 were obtained from National Agriculture Research Centre (NARC), Islamabad. The seeds were sown in earthen pots measuring 27 x 30 cm^2 filled with clay loam and farm yard manure (FYM) in the ratio of 7:1 under natural environmental conditions. Following treatments were made.
T1 = Control
T2 = Biopower (*Rhizobium* + phosphate solubilizing microbes)
T3 = Nitrogen and phosphorus fertilizers
T4 = Biozote (*Rhizobium*)
Biopower and Biozote are trade names of biofertilizers prepared by National Institute of Biotechnology and Genetic Engineering (NIBGE) Faisalabad and National Agriculture

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Research Centre (NARC), Islamabad, respectively. Both biofertilizers were purchased from the respective institutes.

Method of seed inoculation. The seeds of soybean were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 2 min and subsequently washed thrice with sterilized water. The sterilized seeds were moistened in 45 % sugar solution and inoculated with Biozote and Biopower separately and were thoroughly mixed to get a thin, uniform coating of inocula on the seeds. Inoculated seeds were air dried before sowing (Samasegaran *et al.*, 1982).

Application of chemical fertilizers. The chemical fertilizers *viz.* nitrogen in the form of urea and phosphorus as diammonium phosphate (DAP) were applied at the rate of 50 kg/ha. The whole dose of chemical fertilizers was split into four equal sub-doses. The first sub-dose of DAP was applied at the time of sowing while the first dose of urea was applied 10 days after sowing. The remaining 3 sub-doses of both fertilizers were applied subsequently at 10 days interval.

Parameters studied. Number of pods/plant, number of seeds/pod and 100 seed weight were determined. Seed oil content was determined by nuclear magnetic resonance (Robertson and Morrison, 1979). The seed samples were rendered into powder form with grinding mill. The oil was extracted using petroleum ether with Soxhlet (AOAC, 1960) and stored at 4 ºC for further analysis.

Specific gravity (g/cc). Specific gravity of oil was determined using density bottle according to the method described by Pearson (1980).

Acid value (mg KOH/g). Acid value was determined according to the method described by Akubugwo and Ugbogu (2007) and calculated according to the following formula.

Acid value = $[56.1 \times N \times V] / W$
where N = normality of NaOH used; V = volume (ml) of NaOH used, W = weight of the sample used

Free fatty acids (as oleic acid). Percentage of free fatty acids was estimated by multiplying the acid value with the factor 0.503.

Refractive index. The refractive index was determined using refractometer according to the method described by AOAC (1990).

pH value. The oil sample (2 g) was poured into a clean dry beaker and 25 ml of hot distilled water was added to the sample in the beaker and stirred slowly. It was cooled in a water bath to 25 ºC and pH of the sample was recorded.

Biodiesel production. Crude oil was processed to methyl esters (biodiesel) using transesterification method utilizing basic catalyst (Freedman *et al.* 1986). Biodiesel was washed by spraying of water on top of the column at low velocity. Thereafter it was dried in rotary evaporator at 120 rpm at 60°C for 40 min. Methyl ester so produced was determined on w/w (%) basis of ester to soybean oil content.

Statistical analysis. The data was analysed statistically by Analysis of Variance technique (Steel and Torrie, 1980) and through Duncan's Multiple Range Test (DMRT), comparison was made among treatment means (Tables 1 and 2).

Results and Discussion

Number of pods per plant, number of seeds per pod and 100 seed weight (g). The results (Table 1) revealed that chemical fertilizers were highly effective in increasing the number of pods per plant. However, Biopower and Biozote treated plants also showed significant increase in the number of pods per plant as compared to the control. All the treatments resulted in non-significant increase in the number of seeds per pod and 100 seed weight as compared to the control. Asad *et al.* (2004) reported that application of Biopower and Biozote increased the number of pods, number of seeds per pod and seed weight in mung bean as compared to the control. This may be due to the increased rate of nitrogen fixation by biofertilizers which resulted in more nitrate supply to reproductive parts causing dry matter accumulation in seeds leading to increased seed weight in soybean. Shehata and El-Khawas (2003) reported that yield characters such as number of seeds per head, weight of seed per head and weight of 1000 seed in sunflower were significantly increased by the application of each of the two biofertilizers, Biogien and Microbien (trade names), either separately or in combination. Urea has also been reported to increase the yield in sesame (Paul and Savithri, 2003). It was found that the chemical fertilizers and the biofertilizers both have significant effect on the increase in soybean plant growth. It had been estimated previously that biofertilizers could replace utilization of 50 % of the chemical fertilizers (El- Kholy and Gomaa, 2000) without decreasing the green and dry fodder; this could be attributed to the plant growth-promoting substances produced by the biofertilizers (Gomaa, 1995; Bottini *et al.*, 1989).

Seed oil content. All the treatments had stimulatory effect on the seed oil yield. Chemical fertilizer and Biozote treatments significantly increased seed oil content by 32 % and 22 %, respectively, as compared to the control. Biopower treatment exhibited non-significant increase in the seed oil content (Table 1). Similar results were obtained by Sawan *et al.* (2006) who reported that application of chemical fertilizers such as phosphorus increased the seed oil content and oil yield per hectare over the control in cotton. This may be attributed to the role of phosphorus as coenzyme involved in energy transfer reaction so that energy is trapped during photosynthesis in the form of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP), which is subsequently used in the photosynthetic fixation of CO_2 and the synthesis of lipids and other essential organic compounds (Taiz and Zeiger, 1991). Mekki and Ahmed (2005) reported that plants treated with biofertilizers singly or in combination with organic manure + yeast resulted in an increase in the seed oil contents of soybean.

Specific gravity of oil. Chemical fertilizers significantly increased specific gravity of oil by 6% as compared to the control (Table 2). Biopower and Biozote exhibited no significant effect on oil specific gravity. Cetane number, heating value, fuel storage and transportation are important qualities of biodiesel, closely related to the specific gravity (Yuan *et al.*, 2004). Fuel density is correlated with particulate emissions and increase in the density gives increased particulate emissions (Mulin, 1994). It was found that chemical fertilizers increased oil specific gravity but biofertilizers had no effect. Oil obtained from the seeds of biofertilizer treated plants can be utilized successfully for production of good quality biodiesel with low particulate emissions of lighter quality.

Acid value (mg KOH/g) and free fatty acid contents (% FFAs as oleic acid). Results presented in Table 2 reveal that biofertilizers significantly decreased the oil acid value and free fatty acid content as compared to the control. Maximum reduction in oil acid value and free fatty acid content was recorded in the Biopower treatment. Biofertilizers have been reported to be involved in the production of phytohormones such as indole-3-acetic acid (IAA), gibberillic acid (GA3) and ethylene (Bashan *et al.*, 2004), zeatin (Tien *et al.*, 1979), abscisic acid (ABA) and production of plant growth regulatory substances such as polyamines (Thuler *et al.*, 2003). Release of these growth-promoting substances in the soybean plants by biofertilizers may be responsible for altering the physiology of fat metabolism leading to decrease in acid value. Oilseed crops require large amounts of nitrogen as the component of plant proteins, amino acids, nucleotides, nucleic acids and chlorophyll. Adequate supply of nitrogen and phosphorous may have led to increase the supply of nutrients to reproductive parts that led to decrease the seed oil acid value. The higher the acidity of the oil, the smaller is its conversion efficiency to biodiesel. These free fatty acids react with the alkaline catalyst to produce soaps instead of esters. For the completion of alkali-catalysed reaction, free fatty acid (FFA) content lower than 3% is needed (Dorado *et al.*, 2002). At

Table1. Effect of biofertilizers (Biopower and Biozote)and chemical fertilizers (urea and DAP) on number of pods/plant, number of seeds/pod, 100 seed weight and seed oil content of soybean (*Glycine max* L.) and statistical analysis

Treatments	No. of pods/plant	No. of seeds/pod	100 seed weight (g)	Seed oil content (%)
Control	13.00 c	2.183 a	1.500 a	16.29 c
Biopower	16.67 b	2.340 a	1.563 a	17.75 bc
Chemical fertilizers	26.67 a	2.287 a	1.320 a	21.56 a
Biozote	17.00 b	2.200 a	1.600 a	19.92 ab
LSD (5%)	3.216	1.882	1.082	1.8822
F-value*	35.124**	0.0164 ns	0.04629 ns	16.249***

*F-values are from one-way ANOVA; **significant at $p<0.001$; ***significant at $p<0.01$; ns= non-significant

Table 2. Effect of biofertilizers (Biopower and Biozote) and chemical fertilizers (urea and DAP) on specific gravity, pH, acid value, free fatty acid and refractive index of soybean (G*lycine max* L.) oil and statistical analysis

Treatments	Specific gravity (g/cc)	pH	Acid value (mg KOH/g)	Free fatty acid (% oleic acid)	Refractive index
Control	0.92 b	5.00 a	2.63 a	1.31 a	1.46 a
Biopower	0.93 b	4.70 a	1.45 c	0.61 b	1.47 a
Chemical fertilizers	0.98 a	4.63 ab	1.99 b	1.00 a	1.47 a
Biozote	0.94 b	4.30 b	1.46 c	0.73 a	1.46 a
LSD	0.0595	0.3812	2.656	0.05954	0.3812
F-value*	3.763**	6.061**	12.096**	3.856**	6.102 ns

*F-values are from one-way ANOVA; **significant at $p < 0.05$; ns = non-significant higher temperatures, free fatty acids react with metals like zinc, lead, manganese and cobalt etc. This could lead to increased engine wear (Romano, 1982). Addition of more sodium hydroxide catalyst compensates for higher acidity but the resulting soap causes an increase in viscosity or formation of gels that interferes in the reaction as well as with separation of glycerol (Freedman *et al.*, 1984). Reduced oil acid value in biofertilizers is favourable for the production of good quality biodiesel at lower catalyst cost with higher yield.

pH. Biozote treatment significantly decreased the pH of oil as compared to the control. Chemical fertilizers and Biopower were ineffective in altering the pH. Acidity of vegetable oil is correlated with free fatty acids (Kusdiana and Saka, 2001). In the base-catalyzed method of biodiesel production, the quantity of catalyst used depends very much on the acidity of the vegetable oil. More quantity of basic catalyst is required in case of more acidic oil to neutralize the pH. Alkaline catalysts are deactivated by the presence of large amounts of free fatty acids and then excessive amount of alkali is required for the formation of emulsions; and increase in the viscosity ultimately leads to the formation of gels and is associated with problems in glycerol separation and loss in the yield of methyl esters (Crabble *et al.*, 2001).

Refractive index. All the treatments exhibited non-significant variations in the refractive index of seed oil. Nonetheless, maximum increase in the refractive index occurred with chemical fertilizer and Biopower treatments. It has been found that refractive indices of natural fats and oils are related to their unsaturation in an approximately linear way (Rudan-Tasic and Klofutar**,** 1999). This increase in refractive index of soybean seed oil may be due to the increase in concentration of unsaturated fatty acids by Biopower treatment. Biopower exhibited stimulatory effects on the refractive index of the oil and thereby improving the oil quality, which can be utilized for production of good quality biodiesel, particularly for usage in colder regions.

Methyl ester content. Maximum ester content (82%) was obtained from the Biopower-treated oil while 78 % from Biozote-treated and 79% from chemical fertilizer-treated oil (Fig. 1). In the transesterification of vegetable oils, a triglyceride reacts with a short chain alcohol in the presence of a catalyst (base or acid), producing a mixture of fatty acid alkyl esters and glycerol (Schuchardt *et al.*1998). The methyl ester content produced after transesterification depends upon the type of feedstock, catalyst formulation, catalyst concentration, alcohol to oil ratio and reaction temperature. Free fatty acid content in the reactant mixture plays important role in the biodiesel yield (Refaat *et al.*, 2008). The higher yield of methyl

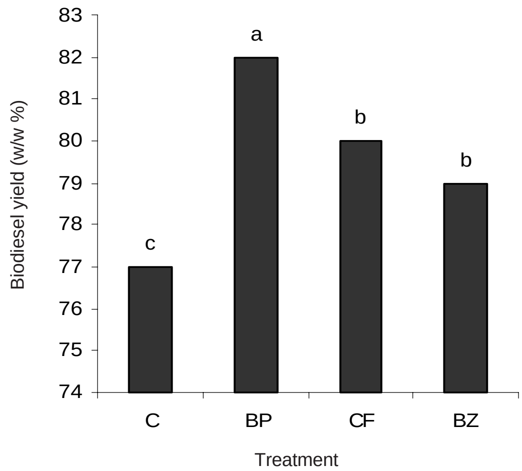

Fig. 1. Effect of biofertilizers (Biopower and Biozote) and chemical fertilizers (urea and diamonium phosphate) on yield of biodiesel.
C=control; BP=biopower; CF=chemical fertilizers; BZ=Biozote

esters from Biopower treated seeds might be due to decrease in free fatty acid content by Biopower. Therefore, it can be inferred that application of biofertilizers and chemical fertilizers significantly increase the overall yield, seed oil content and quantity and quality of biodiesel from soybean. Although chemical fertilizers lead to increase in the yield and soybean oil content but the oil quality decreases due to increase in the specific gravity of oil. On the contrary, biofertilizers not only improve the oil content but also the quality of soybean oil. Thus it can also be inferred that biofertilizers can be supplemented with chemical fertilizers to improve the yield, content and quality of oil, which can be utilized for production of good quality biodiesel on commercial scale. This approach of using microbes is economical, sustainable and environment friendly as biofertilizers have been reported to replace 50% chemical fertilizers (El- Kholy and Gomaa, 2000).

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